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Head injury is not a risk factor for multiple sclerosis: a prospective cohort study

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Background The idea of physical trauma being involved in the causation of multiple sclerosis (MS) had been discussed since the earliest description of the illness. Despite the ongoing debate, the proposed association between physical and especially head trauma and MS failed to be proved or to be refuted conclusively.

Objective To determine whether head trauma is associated with an increased risk of developing MS.

Method A cohort of 150,868 subjects, 95,111 men, and 55,757 women registered in the National Danish Patient Registry with hospital admission for cerebral concussion, contusion, or skull fracture between 1977 and 1992, aged under 55, was selected. This trauma cohort was linked with the Danish MS Registry and followed up to the end of 1999 to retrieve subjects who had onset of MS after the year of the head trauma. We calculated the expected number of subjects, who, under a null-hypothesis, would subsequently develop MS, by using population age-, year-, and sex-specific MS-incidence densities from the Danish MS Registry.

Results For men and women combined, the observed to expected number of MS cases (possible cases included) with onset after the head injury was 182/193.6 (standardized incidence ratio [SIR], 0.94; 95% CI, 0.81–1.09) and for possible MS excluded, 171/164.7 (SIR, 1.04; 95% CI, 0.89–1.21). In an analysis of a sub-cohort of 16,425 subjects with severe trauma (contusion, traumatic cerebral hemorrhage, and base or skull fracture), the observed to expected numbers, including possible MS, were 15/15.3 (SIR, 0.98; 95% CI, 0.55–1.62) and with possible MS excluded, 13/12.9 (SIR, 1.01; 95% CI, 0.53–1.73). As for the total group and for any of the subgroups and for men and women separately, none of the SIRs differed statistically significantly from unity. Neither were there any trends, which could be missed by type II errors.

Conclusion Head injury of any severity does not affect the risk of acquiring MS later in life. *Multiple Sclerosis* 2009; **15:** 294–298. http://msj.sagepub.com

Key words: craniocerebral trauma; epidemiology; head injury; head trauma; multiple sclerosis; pathogenesis; trauma

Introduction

The role of trauma in causing or aggravating multiple sclerosis (MS) has been discussed for many years [1,2]. Although the exact pathogenesis of MS is not completely understood, it is generally considered to be an immune-mediated inflammatory disease of the central nervous system occurring predominantly in genetically susceptible individuals, precipitated by one or more environmental agents, probably of infectious nature. Several risk factors have been correlated with MS prevalence worldwide. However, few published studies have investigated whether head trauma predisposes to MS [3,4]. Clarification of the role of head trauma in MS may have important pathophysiological and legal implications.

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Most publications on trauma and MS have been interview-based case-control studies and case reports. Case-control studies have led to contradicting results [5,6]. As dependent on the patient's memory, case-control studies may be subject to recall bias. A number of these case-control studies have been negative [7,8], but they have generally been too small to resolve the hypothesis satisfactorily.

Prospective cohort studies provide the strongest design to test for a causal relationship between exposure and a disease.

In this article, we identify all cases of MS in a large, nationwide- and population-based cohort of persons admitted to a hospital with head trauma diagnosis.

Patients and methods

We used a cohort of all individuals, who had been discharged from any Danish hospital between 1977 and 1992 with a diagnosis of head injury (ICD8 800.99 to 801.09; 803.99 to 853.99). The cohort was based on files in the Danish National Patient Registry, which is a complete database of all hospital admissions in Denmark since 1978, in which cases are registered by full personal identification, date of discharge, and ICD code of diagnosis by discharge. We have reported on this cohort in a previous publication and briefly summarized the material below [9]. The cohort had been followed up until 1999 to vital status and exact date of death by linkage to the public Civil Registration System, which has been in operation since April 1, 1968. All residents in the country have been assigned a unique personal identification number, including information on date of birth and sex.

From this cohort, we sampled all cases with age less than 55 years at the time of head trauma. Thus, 150,868 individuals (95,111 men and 55,757 women) were included. Another 1,009 individuals with the diagnosis "other or unspecified intracranial lesion" (ICD8 854.99) were not included due to insufficient definition of the injury.

To identify all cases that developed MS subsequent to the trauma up to the time of follow-up, the cohort was linked with the Danish MS Registry that is a nationwide- and population-based registry of all incident cases of MS. It was formally established in 1956 but started operating in 1949 in continuation with a nationwide prevalence survey [10]. Since then, the Registry has continued collecting data on virtually all new and old cases of MS or suspected MS from multiple sources [11]. The Registry has reclassified all cases of MS according to the Allison criteria [12] (cases with onset up to 1993) and to the Poser criteria [13] (cases with onset from 1994). The completeness has formerly been estimated at more than 90% [14].

Statistical analysis

We linked the trauma cohort with the Danish MS Registry to identify subjects with onset of MS from the calendar year after the year of head trauma up to the following events: death, emigration, onset of MS, or end of the year 1999, whichever came first. Follow-up was limited to 1999 as vital status for the cohort was only available up to this year.

To calculate the expected number of MS subjects in the cohort, under a null hypothesis, we used year-, age- and gender-specific incidence density estimates of MS based on the Danish MS Registry.

In the trauma cohort, we also found 114 prevalent cases of MS who had had clinical onset before the year of injury. These prevalent cases were excluded from the cohort under study and did not contribute with person-years in the statistical analyses.

MS patients were classified with regard to certainty of diagnosis. In the "All MS" group, possible cases were included, however not encompassing "observational cases" (typically CIS in which the diagnosis was never upgraded) or cases with poor documentation. The definite and probable MS includes both the Poser classes, clinically definite, laboratory supported definite, and chronic progressive MS [13], and the Allison classes, clinically definite, probable, and early probable MS. These filters were used both with retrieving and counting observed MS cases and calculating the expected numbers.

Figure 1 illustrates that 80% were under 35 years and 96% under 50 years, at the time of injury.

There was a distinct male preponderance. Concerning the distribution of diagnosis of head trauma, a larger part of the patients (134,442) had been taken to hospital for cerebral concussion representing mild-to-moderate head trauma, whereas 16,426 had suffered severe head trauma (contusion of the brain, traumatic brain hemorrhage, or skull fracture). In a subgroup analyses, we included only these cases.

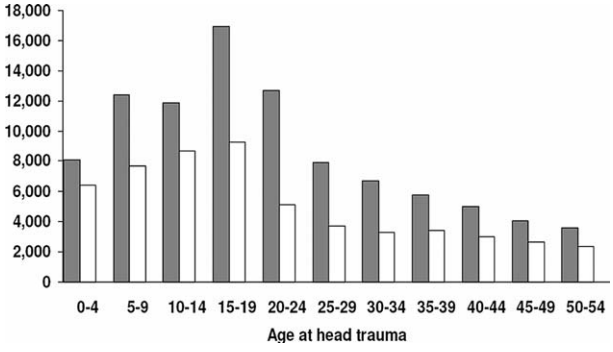

Figure 1 Number of trauma cases by sex and age at time of injury. White bars, females; Shaded bars, males.

Table 1 All head injuries

	Men + women		Men		Women	
	All MS[a]	Possible cases excluded	All MS[a]	Possible cases excluded	All MS[a]	Possible cases excluded
Observed	182	171	80	74	102	97
Expected	193.6	164.7	95.1	474.2	98.5	90.5
Person years observed	2,178,931		1,371.088		807,843	
SIR	**0.94**	**1.04**	**0.84**	**1.00**	**1.04**	**1.07**
(95% CI)	(0,81–1.09)	(0.89–1.21)	(0.67–1.05)	(0.78–1.25)	(0.84–1.26)	(0.87–1.31)

MS, multiple sclerosis; SIR, standardized incidence ratio.

Results

The trauma cohort of 150,868 people had been followed for a total of 2,178,921 person-years. The average observation time per patient was 14.4 years (range, 1–22 years). Among the members of this cohort, we retrieved 182 cases from the Danish MS Registry, who had clinical onset of MS at the earliest in the calendar year after the year of trauma and at the latest during 1999; this number being of the same magnitude as the expected number of 193.6 cases, calculated from the sex-, age-, and calendar year-specific incidence densities of MS in the Danish population. The standardized incidence ratio (SIR) was 0.94 with 95% CI, 0.81–1.09. Table 1 illustrates that in no instances the observed numbers even showed a weak trend let alone statistical significant deviations from the null-hypothesis-derived expected values. Even when restricting the analyses to people with severe head trauma and excluding cases with possible MS from the numerator as well as from calculation of expected numbers, the null-hypothesis remained totally unchallenged (Table 2).

Discussion

The proposed association between head injury and onset of MS has been examined in numerous studies, with contradicting results.

From 1897, where Mendel reported four cases of MS beginning within a year of severe skull or spinal injury [15], several studies have been published, mostly studies of anecdotal character or case-reports, showing an apparent association between trauma and MS.

In 1902, Hoffman reported 8 of 100 cases with new-onset MS (8%) within one year after experienced trauma [16], and Von Hoesslin found antecedent trauma occurring within the previous two months in 58 of 516 (11.2%) new-onset cases of MS [17]. The first controlled positive study was published in 1952 by McAlpine, who obtained a history of trauma 3 months preceding the emergence of the first symptom of MS in 36 of 250 patients (14.4%) compared with only 13 (5.2%) of the controls [6]. As a curiosity, 20 of 36 MS patients had their first symptom of MS in the extremity previously injured, but some fundamental concerns about this study regarding possible recall bias, comparability of the cohort groups and lack of details on the trauma included has been raised [18].

In 1954, Kurland [19] conducted a population-based case-control study including 112 MS patients and 123 control subjects carefully selected from the same community. They found no significant association between head injuries causing unconsciousness and the onset of MS, indicating that head trauma was not a risk factor for MS.

In 1993, Siva, *et al*. [4] executed a population-based record linkage study of MS onset following

Table 2 Severe head injuries

	Men + women		Men		Women	
	All MS[a]	Possible cases excluded	All MS[a]	Possible cases excluded	All MS[a]	Possible cases excluded
Observed	15	13	11	10	4	3
Expected	15.3	12.9	8.4	6.5	7.0	6.4
Person years observed	200,959		137,559		63,400	
SIR	**0.99**	**1.01**	**1.31**	**1.53**	**0.58**	**0.47**
(95% CI)	(0.55–1.62)	(0.53–1.73)	(0.65–2.36)	(0.73–2.83)	(0.15–1.49)	(0.09–1.39)

[a]In the "All MS" group, possible cases were included, however *not* encompassing "observational cases" (typically CIS in which the diagnosis was never upgraded) or cases with poor documentation.
MS, multiple sclerosis; SIR, standardized incidence ratio.

head injury. In a cohort of 819 head injury cases (age 10–50 years) from the Olmsted County population, none developed MS within 6 months of the trauma. Their definition of trauma included head trauma severe enough to produce skull fracture, loss of consciousness, focal neurological deficits, or posttraumatic amnesia. Goodin, *et al*. [18] regarded this study as strong Class II evidence, despite lacking statistical power, for restricting any posited risk of MS after serious head trauma to no more than 1.3%.

Recently, Goldacre, *et al*. [3] undertook a population-based record linkage study of 110,993 individuals in a hospital-based head injury cohort. They compared this cohort to a cohort of 534,600 patients from the same hospitals, treated for other conditions within the same time frame, as to the number being affected with MS after the injury within the follow-up period. The use of hospital controls somehow limits the reliability of the findings because one cannot exclude that these controls constitute a selected group compared with the overall population. In line with the findings of our study, the authors did not observe that head injuries were associated with onset of MS neither for short or long-term follow-up, irrespective of the severity of the trauma.

Some of the studies [6,15–17] have suggested that MS is likely to occur within months of the head trauma. As onset of MS is determined with an accuracy of just one calendar year, a hypothesized abundance of onset of MS immediately after the injury could be missed, as we only counted cases with onset of MS at the earliest in the calendar year following the year of the injury. However, we also identified 17 cases with onset in the very same calendar year as the head injury was diagnosed, in which 10.43 cases would be expected. The SIR for the year of injury is thus 1.63 (95% CI, 0.95–2.62). Statistically, 50% of the 17 cases may have occurred before the injury. This insignificant abundance may be attributed to increased attention to symptoms after the injury, parallel to women who discover a lump after a minor breast injury.

It is meaningful to analyze the subgroup of severe head trauma separately because it is a reasonable presumption that any association between trauma and MS should be most apparent for more serious traumatic injury because this would be expected to produce the greatest breakdown of the blood-brain barrier or exposure of the immune system to self-antigens [18].

However, in the present study, neither the analysis of the subgroup of 16,393 patients, subjected to severe head trauma, nor of the whole group of 150,868 patients, also including the milder trauma, indicated an increased risk of MS later on.

By its design, the present study has avoided selection and recall bias, and it is adequately sized for acceptable statistical power; indeed it includes all diagnosed cases of head trauma in a whole country over more than two decades. The data included are purely established for administrative reasons years before the hypothesis was established excluding information bias. In general, studies of cohorts with a specific exposure, who are followed over time for MS as a specified endpoint to occur, provide the best opportunity to test hypotheses concerning disease etiology because they avoid the biases that are normally associated with case-control studies. Because years or even decades may have elapsed before MS break out in the exposed and non-exposed index persons, such studies are usually only to be performed as historical cohort studies using exposure cohorts formed in the past, probably without any thought on the endpoint in question. The present historical cohort study confirms that head injury does not cause or precipitate MS. The important question whether head injuries aggravate an already manifested MS remains unanswered by this study due to its design but will be an interesting topic of a future study.

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References

1. Kurland, LT. Trauma and multiple sclerosis. *Ann Neurol* 1994; **36**(Suppl): S33–S37.
2. Poser, CM. The role of trauma in the pathogenesis of multiple sclerosis: a review. *Clin Neurol Neurosurg* 1994; **96**: 103–110.
3. Goldacre, MJ, Abisgold, JD, Yeates, DG, Seagroatt, V. Risk of multiple sclerosis after head injury: record linkage study. *J Neurol Neurosurg Psychiatry* 2006; **77**: 351–353.
4. Siva, A, Radhakrishnan, K, Kurland, LT, O'Brien, PC, Swanson, JW, Rodriguez, M. Trauma and multiple sclerosis: a population-based cohort study from Olmsted County, Minnesota. *Neurology* 1993; **43**: 1878–1882.
5. Alter, M, Speer, J. Clinical evaluation of possible etiologic factors in multiple sclerosis. *Neurology* 1968; **18**: 109–116.
6. McAlpine, D, Compston, N. Some aspects of the natural history of disseminated sclerosis. *Q J Med* 1952; **21**: 135–167.
7. Jellinek, EH. Trauma and multiple sclerosis. *Lancet* 1994; **343**: 1053–1054.
8. Lauer, K. Physical trauma and multiple sclerosis. *Neurology* 1994; **44**: 1360–1364.
9. Inskip, PD, Mellemkjaer, L, Gridley, G, Olsen, JH. Incidence of intracranial tumors following hospitalization for head injuries (Denmark). *Cancer Causes Control* 1998; **9**: 109–116.

10. Hyllested, K. Disseminated sclerosis in Denmark: prevalence and geographical distribution (Doctoral thesis). Copenhagen: J. Jorgensen; 1956.
11. Koch-Henriksen, N. The Danish Multiple Sclerosis Registry: a 50-year follow-up. *Mult Scler* 1999; **5**: 293–296.
12. Millar, JH, Allison, RS. Familial incidence of disseminated sclerosis in Northern Ireland. *Ulster Med J* 1954; **23**: 29–92.
13. Poser, CM, Paty, DW, Scheinberg, L, McDonald, WI, Davis, FA, Ebers, GC, *et al*. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann Neurol* 1983; **13**: 227–231.
14. Koch-Henriksen, N, Hyllested, K. Epidemiology of multiple sclerosis: incidence and prevalence rates in Denmark 1948–64 based on the Danish Multiple Sclerosis Registry. *Acta Neurol Scand* 1988; **78**: 369–380.
15. Mendel, K. Tabes und multiple Sklerose in ihren Beziehungen zum Trauma. *Dtsch Med Wochenschr* 1897; **7**: 97–100.
16. Hoffman, J. Die multiple Sklerose des Centralnervensystems. *Dtsch Z Nervenheilkd* 1902; **21**: 1–27.
17. Von Hoesslin, R. Ueber multiple Sklerose. Muenchen: JF Lehmanns Verlag; 1934. p. 68–74.
18. Goodin, DS, Ebers, GC, Johnson, KP, Rodriguez, M, Sibley, WA, Wolinsky, JS. The relationship of MS to physical trauma and psychological stress: report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. *Neurology* 1999; **52**: 1737–1745.
19. Kurland, LT. Epidemiologic factors in the aetiology and prognosis of multiple sclerosis. *Ann NY Acad Sci* 1954; **58**: 682–701.